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INTRODUCTION

In June 2006, I applied for a final no-cost extension to complete Task 2c of the project which required more time than previously anticipated. Progress was slow owing to difficulty in recruiting a qualified laboratory technician for a single year. During the spring and summer (2006), I trained an undergraduate student, Ms. Giselle Burnett, who is now assisting me with cell culture, western blotting and RT-PCR. The project will be completed in the current year (06/07).

BODY

STATEMENT OF WORK

Tamoxifen and tumor-associated macrophages

Task 1. Determine the effect of *in vitro* co-culture on gene expression in BC cells and THP-1- macrophages (Months 1 - 18):

(a) Recruitment of postdoctoral fellow (Months 1 - 2)
(b) Grow cells, set up co-cultures of BC and THP-1 macrophages (Months 1 - 7)
(c) Isolate mRNA for gene expression array analysis, Months 4 - 12
(d) Standardize and calibrate gene expression arrays for proliferation-related gene expression in BC cells co-cultured with THP-1 macrophages (Months 11 - 18)

Task 2. Studies on effects of anti-inflammatory and macrophage-modulating compounds on macrophage and BC gene expression (Months 16 - 36):

(a) Co-culture of cells for studies on the effects of anti-inflammatory agents (Months 16 - 30)
(b) Isolation of mRNA for gene RT-PCR, and gene expression arrays for Task 2 (Months 17 - 30)
(c) Western blotting, ELISA for cytokines, RT-PCR (Months 24 - 36)

To date we have observed that when macrophages and breast cancer cells are co-cultured there is reciprocal regulation of gene expression in both cell types. Our data suggest that of the many cytokine and other genes whose levels of expression are altered when breast cancer cells and macrophages are grown together, *HIF-1α* and *MIF* (macrophage migration inhibitory factor) showed significant changes that may influence macrophage function in relation to tumor progression. We have analyzed *HIF-1α* gene expression by gene arrays and RT-PCR, and confirmed that HIF-1 is constitutively expressed in THP-1 monocytes and THP-1-derived macrophages. Furthermore, we have shown by TransAM™ ELISA, a transcription factor binding assay that measures the functionality of the HIF-1 heterodimer by its ability to bind a hypoxia response element (HRE) consensus sequence (5'-RCGTG-3'), that functional HIF-1 is expressed in THP-1 monocytes, and moderately up-regulated by 50 μM cobaltous chloride, a hypoxia mimic (Fig. 1). HIF-1α is also expressed in MCF-7, both under normoxia and hypoxia. Recent reports in the literature support a critical role for HIF-1 in breast cancer progression, because CXCR4, a chemokine receptor associated with metastasis [1], is a direct transcriptional target of HIF-1 [2]. CXCR4, a G-protein-coupled receptor, is activated upon binding to its ligand SDF-1/CXCL12 and stimulates several signal transduction pathways that regulate motility, chemotaxis, survival and proliferation of many cell types [3,]. Paradoxically, in our experiments, HIF-1α expression is extinguished in THP-1 macrophages when the two cells are co-cultured (Fig. 2, A, B). MCF-7 cells, however, continue to express HIF-1α (Fig. 2, C, D). It was recently reported that the human MIF gene contains a HRE in its 5' UTR and is a transcriptional target of HIF-1 [4]. Therefore, MIF expression may be driven by hypoxia through HIF-1. MIF has been implicated in paclitaxel resistance of prostate cancer due to modulation of the multidrug resistance (MDR-1) gene [5].

KEY RESEARCH ACCOMPLISHMENTS

We have observed that:

- THP-1 monocytes and THP-1-derived macrophages produce functional HIF-1 (as opposed to HIF-1α mRNA) under normoxia. Therefore, HIF-1α is not targeted for proteasomal degradation under normoxia in these cells. The implications of this finding for tumor-associated macrophages are being explored.

- Our Western blot analysis of MIF shows that two forms of MIF are present in macrophages and MCF-7 cells, suggesting that MIF is either proteolitically processed or otherwise covalently modified in MCF-7. When MCF-7 is co-cultured with THP-1-derived macrophages, both forms are present in the MCF-7 cells (Fig. 3). Such variation has not been reported in the literature. Also, MIF protein is down-regulated in LPS-activated THP-1 macrophages.

REPORTABLE OUTCOMES

None

CONCLUSIONS

Both HIF-1 and MIF play important roles in the pathogenesis of breast cancer. Their activity and function in tumor-associated macrophages may drive tumor progression. While these proteins may provide growth and survival advantages to TAMs, they may also confer vulnerabilities, such as susceptibility to endogenous or exogenous agents. An exhaustive search for putative susceptibilities in the context of the tumor microenvironment may lead to the identification of new therapeutic targets.

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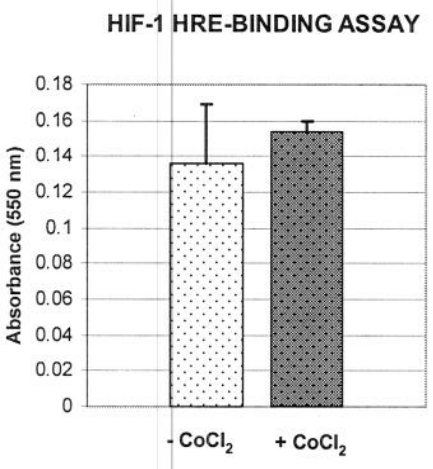

Fig. 1. HIF-1 hypoxia response element-binding assay of control THP-1 monocytes and THP-1 treated with 50 μM $CoCl_2$ for 6 h.

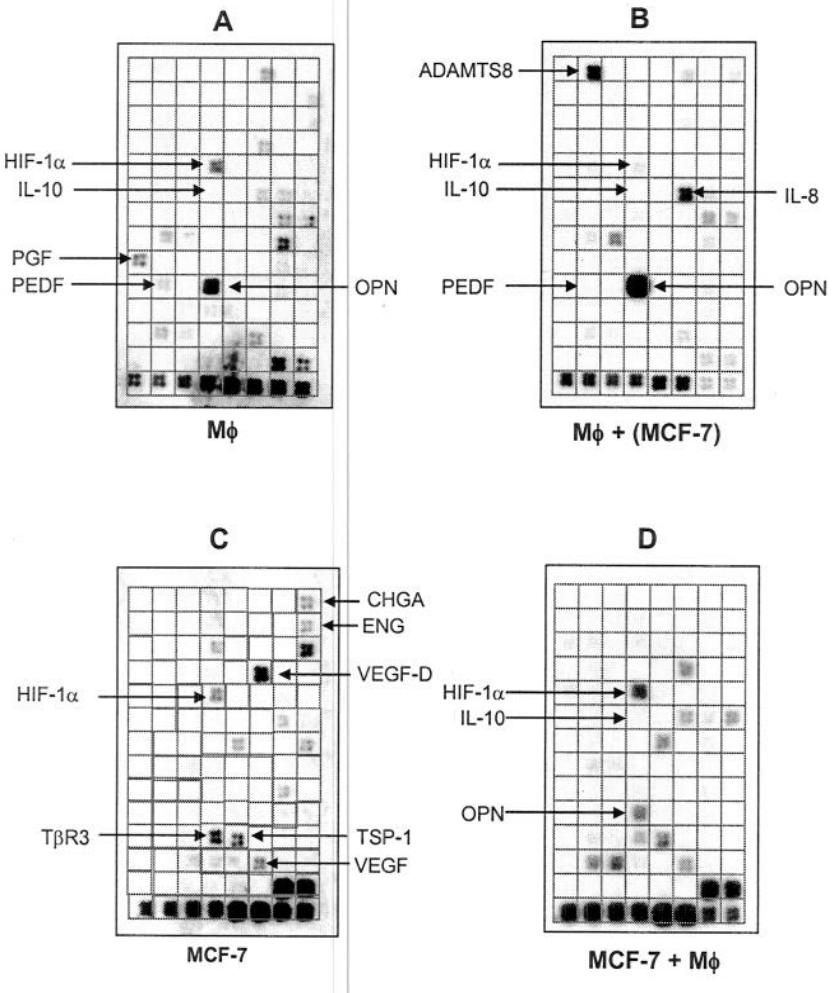

Fig. 2. Angiogenesis related gene expression in A, THP-1-derived macro-phages; B, macrophages co-cultured with MCF-7; C, MCF-7 cells; and D, MCF-7 co-cultured with macrophages.

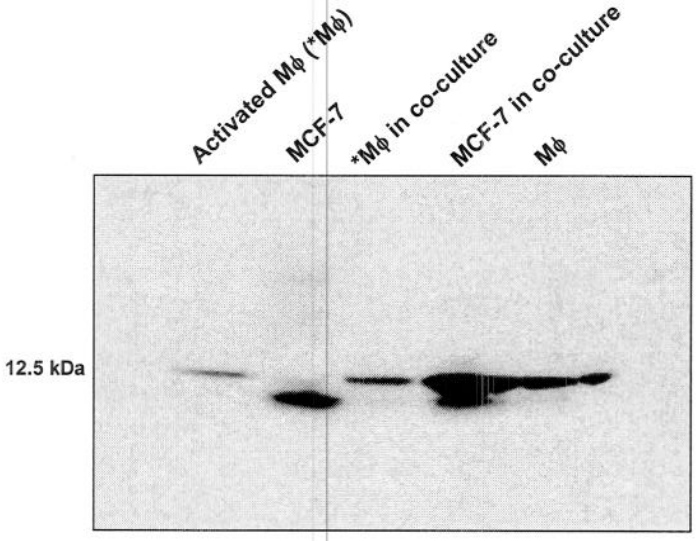

Fig. 1. Western blot of MIF in THP-1-derived macrophages and MCF-7 breast cancer cells. LPS-activated macrophages, MCF-7 cells, activated macrophages co-cultured with MCF-7, MCF-7 cells co-cultured with activated macrophages, and resting macrophages were probed for MIF protein. For each sample, 50 μg lysate protein was resolved in 4-20% SDS-PAGE gradient gels, electroblotteded on to nitrocellulose membrane and probed with rabbit polyclonal anti-human MIF IgG (Santa Cruz Biotechnology; FL-115, sc-20121). Secondary antibody was horse radish peroxidase-conjugated goat anti-rabbit IgG.